

# **Guide for the detection of a Potential Recrudescence during the period of Post Treatment Surveillance (PTS)**

Note: This guide is an adaptation of the original English document produced by the Program Coordinating Committee (PCC). Oepa adapted the guide for national program distribution.

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## List of Terms

|   |   |
|---|---|
| <i>ATP:</i>   | Annual Transmission Potential   |
| <i>C.I.</i>   | Confidence Interval   |
| <i>DNA:</i>   | Deoxyribonucleic acid   |
| <i>EEP:</i>   | In-Depth Epidemiological Evaluation (from the Spanish, Evaluaciones Epidemiologicas en Profundidad)   |
| <i>ELISA:</i>                                       | Enzyme-Linked Immunosorbent Assay; biochemical technique used to detect an antibody or antigen.   |
| <i>EuSIMON:</i>                                     | Euphoria Simulation MOdel of ONchocerciasis; Euphoria program language mathematical model used to predict epidemiological results in endemic communities under different simulated scenarios. |
| <i>IR:</i>  | Infectivity Rate  |
| <i>L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>:</i> | Parasite larval stages found in the fly vector  |
| <i>Mf:</i>  | Microfilariae   |
| <i>MfC:</i>   | Microfilariae in the Cornea   |
| <i>MfCA:</i>  | Microfilariae in the Anterior Chamber of the eye  |
| <i>Ov-16:</i>                                       | Recombinant antigen of <i>O. volvulus</i>   |
| <i>PAHO:</i>  | Pan-American Health Organization  |
| <i>PCC:</i>   | Program Coordinating Committee  |
| <i>PCR:</i>   | Polymerase Chain Reaction; technique in molecular biology to amplify DNA  |
| <i>PEC:</i>   | Potentially Endemic Community   |
| <i>PR:</i>  | Potential Recrudescence   |
| <i>PRE:</i>   | Potential Recrudescence Event   |
| <i>R<sub>0</sub>:</i>                               | Reproduction Ratio; a measure of the parasite population reproductive success   |
| <i>SBD:</i>   | Seasonal Biting Density   |
| <i>STP:</i>   | Seasonal Transmission Potential   |
| <i>WHO:</i>   | World Health Organization   |

## Introduction

Onchocerciasis (river blindness) is caused by the vector-borne parasite *Onchocerca volvulus* and was endemic in 13 foci in 6 countries in WHO's Region of the Americas: Brazil, Colombia, Ecuador, Guatemala, Mexico and Venezuela. The Onchocerciasis Elimination Program for the Americas (OEPA) is a regional partnership that includes the endemic countries, the Pan American Health Organization (PAHO/WHO), The Carter Center, Lions Clubs International and local Lions clubs, the United States Centers for Disease Control and Prevention (CDC), the Bill and Melinda Gates Foundation, several universities, the Mectizan Donation Program and Merck & Co. The goal of the initiative is to provide mass drug administration (MDA) at least twice per year with the safe and effective oral drug, ivermectin (Mectizan®, donated by Merck & Co.). Treatment should reach at least 85% treatment coverage of the eligible population. OEPA is now operating under a 2008 by PAHO's Directing Council Resolution CD48.R12 calling for the regional elimination of ocular morbidity caused by onchocerciasis and interruption of transmission of the parasite by 2012 (PAHO 2008).

In 2001 the World Health Organization published the document "Certification of Elimination of Human Onchocerciasis: Criteria and Procedures" in which is established the different phases to be followed by a country to achieve a certification of elimination of onchocerciasis. Each of these phases is associated with a stage of transmission resulting in the following categories (also see Annex 1):

- 1. Transmission ongoing:** Infective stage larva (L<sub>3</sub>) of *O. volvulus* are found in the vector population (heads) and children less than five years of age are positive for mf in skin, nodules, and serology..
- 2. Transmission Suppressed:** No infective stage larva (L<sub>3</sub>) of *O. volvulus* are found in the vector population (heads) and children less than five years of age are negative for mf in skin, nodules, and serology. All indicators show a lack of transmission in the area. The next step is for the PCC to recommend to the National Ministry of Health the suspension of treatment and the start of Post Treatment Surveillance.
- 3. Transmission Interrupted:** The PCC, after careful analysis of indicators, recommends to the Ministry of Health the suspension of treatment in the particular focus and to start Post Treatment Surveillance. The Ministry of Health accepts the recommendation.
- 4. Transmission Eliminated:** After three years of Post Treatment Surveillance (PTS), the indicated evaluations for this period have been carried out and all the results confirm a continued interruption of transmission.

This guide was developed by the PCC and OEPA as a field document that has as its focus the three year period defined by Post Treatment Surveillance (PTS) and describes the activities that distinguish and bridge category 3 (Transmission Interrupted) and category 4 (Transmission Eliminated).

## **Role of the PCC**

The Program Coordinating Committee (PCC) is the technical steering committee of OEPA and makes recommendation to OEPA staff and to participating countries pertaining to the process of onchocerciasis elimination in each of the region's 13 foci with respect to each of the stages of transmission described.

The PCC should be engaged in this process up until the official request by the country to WHO for certification by an international certification team (WHO guidelines, 2001). The PCC should formally review the results of the PTS activities with the national ministry of health team within six months of the data having been collected. If there is no evidence of recrudescence, then the PCC will issue a written opinion to the government that elimination has been achieved and, if the last focus in the country, suggesting that the government request that the final WHO certification process begin. However, as with the decision to halt interventions prior to PTS, it is the government's decision to accept or not the interpretation of their PTS results by PCC, and whether to accept the PCC recommendations related to the country request for WHO certification.

## **Origins of the 'Post Treatment Surveillance' concept**

The idea of post treatment surveillance originated in the 2001 World Health Organization document "Certification of Elimination of Human Onchocerciasis: Criteria and Procedures" in which a three year period was recommended that was called the "Pre-Certification Period." This was a "national period" (i.e., related to the entire country rather than individual onchocerciasis foci) during which surveillance should be instituted to detect recrudescence of transmission of *O. volvulus* after all nation-wide interventions have been halted.

*"With the ceasing of interventions, a 3 year pre-certification period would start. At the end of this pre-certification period, it must be shown that, although intervention has ceased, no new incident onchocerciasis cases have been registered and no infected vectors identified." (WHO, page 12)*

Based on this original WHO statement, the PCC modified the original concept of a national "pre-certification period" to be applied to foci rather than entire countries. This concept (a post treatment surveillance period rather than a pre-certification period) was necessary because of the focal nature of the infection in the Americas, and the need to follow the progress of each focus through the four phases of elimination outlined above.

## **Definition of PTS**

The PCC defines Post Treatment Surveillance in the following manner:

"Post Treatment Surveillance (PTS) is a 3-year period that begins with the termination of ivermectin mass treatment for onchocerciasis. At the end of this period, it must be documented that, although intervention has ceased, no evidence of recurrent transmission has occurred based on PCR testing for *O. volvulus* DNA in a substantial

sample of vectors. Should positive entomological results be found, then serologic antibody testing in children less than ten years of age in the endemic area, through the ELISA Ov-16 antigen should be undertaken. Positive results may be confirmed if required using PCR testing of skin snips, in accord with the accepted criteria. If the data indicate no recrudescence of *Onchocerca volvulus* transmission, then the infection can be declared eliminated. Post elimination (also termed “post endemic”) surveillance may continue in formerly endemic foci beyond the initial 3 year PTS if deemed necessary.”

### **Principles of PTS**

1. The evaluations comprising PTS should not be completely new to the programs but continuations of previous programmatic field impact evaluations;
2. Indices obtained during PTS would therefore be comparable with ‘stop treatment’ surveys, the latter serving as the “PTS baseline;”
3. If a potential recrudescence event is detected, the PCC should be consulted immediately, and before any other actions are taken;
4. The response to a potential recrudescence event (PRE) should involve flexibility based on the transmission dynamics, along with other characteristics, of the focus being considered.
5. In addition, further studies may be requested that are similarly tailored to the focus and the nature of the potential recrudescence “signals” emerging from the initial PTS studies.
6. The EuSIMON mathematical model, as it becomes available, should be consulted to test the validity of a potential recrudescence event under the epidemiological and entomological conditions of the focus. Modeling activities should include interpretation of PTS results and subsequent studies relative to breakpoints,  $R_0$  projections, statistical certainty of multiple simulations, and risk for slow versus explosive recrudescence.
7. In the event of a PRE, the PCC should take a measured analytical approach, i.e., careful evaluation before re-initiating mass drug administration. If the PRE is judged to be a true recrudescence, then a prompt programmatic response will be devised in consultation with the national program.
8. Prompt programmatic response should include state-of-the-art methods specific to the affected focus, such as, vector control, increased frequency of treatments with ivermectin, nodulectomy, and use of doxycycline.
9. Care should be taken using new tools as yet untested in the Americas (research) as “evidence” for recrudescence until such tools have been fully evaluated and validated for the Region.
10. In case of doubt or need of clarification regarding any of the guidelines presented in this document, contact the PCC and OEPA.

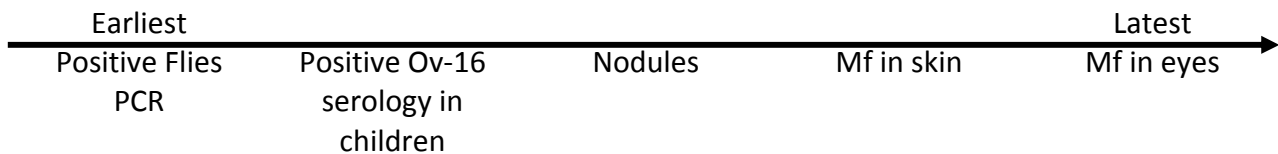
### **Continuation of programmatic activities during PTS**

After the suspension of treatment, programmatic activities, now in the form of PTS, must continue for a minimum of three years, in accord with WHO guidelines. Ministries of Health, political leaders and donors should recognize that the national onchocerciasis programs do not cease field programmatic operations when ivermectin treatments are halted.

## Expected evolution of a recrudescence

The expected evolution of how a potential recrudescence would unfold is based on the natural history of onchocerciasis which requires 18 months from the time of the inoculation of L<sub>3</sub> larvae until the presence of microfilariae in the skin. Given this, the earliest signal of a possible recrudescence would be the presence of infective larvae in the fly; followed by the discovery of antibodies to the antigen Ov-16 in children; followed by the appearance of nodules and then microfilariae in the skin; and finally, as a late manifestation, the appearance of microfilariae in the eyes. This progression allows the process of Post Treatment Surveillance to be considered in the stages that are shown in Figure 1.

**Figure 1: Evolution of Onchocerciasis Recrudescence**



Given this, the most cost effective approach giving the earliest signal of recrudescence with the least inconvenience to the population resident in the focus consists of screening first for positivity in the vectors, followed by screening for antibodies in children less than ten years of age, and finally, confirmation via skin biopsy PCR of those children found to be serologically positive.

## Basic PTS guidelines

The guidelines for PTS issued by the PCC comprise three components:

### Component 1: Educational interventions during the period of PTS

Each national program should guarantee that all communities and all individuals that have been involved in the onchocerciasis elimination process have a clear understanding of both the procedures undertaken to determine that an interruption of transmission has been achieved and the rationale behind the decision to suspend ivermectin treatment. In addition, each national program should work with the teams of health workers, leaders and volunteers, and the communities involved to define the specific activities that are to be undertaken during the three year PTS period, keeping in mind the recommendations put forth by the PCC. Of particular importance will be the need to maintain permanent ties of communication between the various health teams, leaders and volunteers and the communities they serve through the strengthening of health promotional activities and keeping alive community interest and participation in achieving the goal of elimination.

#### 1.1 Educational Activities for Program Personnel

Each national program should guarantee that program workers and local health teams, along with local health agents, leaders and volunteers that have been involved with the program, are thoroughly informed about the evaluations that have taken place to determine that

transmission has been interrupted. The results of these evaluations should be reviewed jointly so as to provide clarity with regards to the rationale and decision making on the part of the Ministry of Health to suspend treatment with ivermectin in the communities.

The methods to be utilized to accomplish these educational goals should be defined by each national program in accordance to their particular characteristics and previous experience developed in the field of education.

### **1.2 Community Educational Activities**

Program workers, local health teams and agents, and community leaders should thoroughly explain to the communities the activities that have taken place, the results of those activities, and the reasoning behind the suspension of treatment with ivermectin.

Educational activities utilized within the community should be defined by each national program in relation to their previous experience.

### **1.3 Central Level Program Accompaniment**

It should be highlighted that central level program accompaniment is fundamental to the successful undertaking of educational activities at all levels of implementation, and ensures that all persons and all communities involved in the program have the necessary information to understand changes in program activities. This is especially true with regards to the suspension of mass treatment with ivermectin. It is important that program workers, local health teams and agents, and community leaders and volunteers know that program coordinators at all levels are present at all activities, in the identification of needs, and encouraging the resolution of problems that are encountered along the way.

## **Component 2: Evaluations during PTS**

If recrudescence of onchocerciasis were to occur during the period of PTS, it would not occur in an abrupt manner (see Figure 1). For this reason, the OEPA Coordinating Committee (PCC) recommends that the participant countries conduct an entomological evaluation during the second and/or third year of the PTS. This evaluation should be conducted during the period of peak transmission and would confirm whether or not transmission continues interrupted and, in effect, whether or not it has been eliminated.

The principle element of the PTS period will be entomological evaluations to determine the presence of parasite DNA in the *Simulium* vectors.

### **2.1 Entomological Evaluation**

(Katholi 1995, Unnasch 1996, Katholi 2002)

- Entomological evaluation by PCR technique. (see Annex 2)
- A minimum of 10,000 flies by focus (if available), collected in sentinel and/or extra-sentinel communities and processed in pools of up to 50 flies by PCR and PoolScreen analysis so as to obtain the infectivity rate. Extra-sentinel villages may be needed to increase focal representation and statistical certainty that transmission is interrupted.

- Timing: Fly collections will be conducted during the peak transmission season for each focus, and initiated no later than Year 3 of PTS. Where the peak of transmission seasons span two years (October-February, for example), collections would need to be launched in Year 2.
- Body pools are analyzed by PCR first and upon finding the first positive body pool (containing larva stages L<sub>1</sub> and L<sub>2</sub>), the analysis is switched to head pools (possibly containing infective stage larva or L<sub>3</sub>).
- To confirm that transmission continues interrupted, or in effect, eliminated, the following results should be obtained:
  - An infectivity rate (L<sub>3</sub> infection in heads) by PCR of <1/1000 (0.1%) in parous flies or <1/2000 (0.05%) in all flies, assuming a 50% parous rate. A 95% CI will be used.
  - An Annual Transmission Potential (ATP) or Seasonal Transmission Potential (STP) under 20 L<sub>3</sub>s per season. Use of ATP/STP is essential in areas where vector biting density is so low that the 95% CI of <1/2000 cannot be demonstrated (Lindblade 2007).

## **2.2 Serological Survey**

(Lobos 1991, Lipner 2006, Lindblade 2007)

- A survey using the ELISA technique to determine the presence of antibodies to the antigen Ov-16 should be conducted in Year 3, only if the entomological evaluation indicates that there is a recrudescence in transmission. (see Annex 3)
- 3,000 children < 10 years of age will be tested via ELISA to detect IgG4 specific antibodies to the recombinant antigen Ov-16.
- The WHO Certification Guidelines ask for a five year cumulative incidence rate of <1/1000 (<0.1%); here, the prevalence of Ov-16 antibodies will be taken as the equivalent to this cumulative incidence rate. Consequently, to calculate a prevalence rate of <0.1% with a 95% C.I., assuming no positives, a sample size of at least 3000 children < 10 years of age is required.
- If this number does not exist within the focus, then as many children in this age group as can be found should be surveyed.
- Sampling should be representative of the entire focus.
- Analysis should allow for stratification by age.

Ov-16 is a circulating pre-patent antigen and antibodies to this antigen indicate exposure and possible pre-patent infection, that is, an infection that is incubating and not a full-blown patent infection. Therefore, if children are found positive by Ov-16 serology, and the value of the indicator is above 0.1%, re-testing by PCR skin biopsy (to determine infection) should be considered. If these serologically positive persons were found negative by PCR, they will then be considered negative for a patent infection with *O. volvulus*, but still could be considered as *O. volvulus* "exposed."

## **2.3 Skin Biopsy PCR in Serologically Positive Children**

Ov-16 serology (using the ELISA technique) detects an exposure to the parasite without being able to determine when that exposure may have occurred, while the technique of PCR establishes whether or not there is an *O. volvulus* infection. To this end, biopsies should be conserved in ethanol or absolute isopropanol for PCR processing.

#### **2.4 Regarding the Age of Children to be Serologically Evaluated**

An issue of the age of children in this evaluation is worth mention here as it was a source of considerable debate within the PCC.

In the criteria for the certification of onchocerciasis elimination, WHO establishes as one of the indicators for the interruption of transmission,

“the absence of detectable infection (evidenced by mf, nodules, immunological tests and other analysis) in children up to 5 years of age that have not received treatment (e.g., those that are becoming eligible for their first dose of ivermectin). A 5 year cumulative incidence rate with less than 1 new case per 1000 susceptible children is acceptable (provided that the appropriate population size is available).”

WHO guidelines sought to calculate a 5 year cumulative incidence of  $<1/1000$ , so that only 5 year old children can provide the “five year cumulative incidence” data sought, because each 4 year old has only a 4 year incidence density experience, each 3 year old a 3 year experience, etc.

However, from an operational perspective, in most endemic countries in the Americas it is difficult to find grouped preschool children under the age of 5 years accessible for sampling. In addition, parents are reluctant to let very young children submit to bloodletting, even if a finger prick.

The PCC recommendation for testing children less than ten years of age would still fulfill the WHO criteria as using older children would provide even stronger support for transmission interruption (i.e., each negative 8 year old contributes 8 year's incidence density to the formula, etc.). Secondly, Ov-16 serology is not affected by ivermectin treatment.

Finally, acquisition of infection rises fastest between 5 and 20 years of age. However, children  $>10$  years of age may misrepresent transmission status since these children could be seropositive from exposure or infection in the distance past, yet transmission could have been interrupted in the area for many years. For the same reason, adults would not be a good indicator age group for serology studies, because of the possibility of persistent antibodies due to exposure to pre-control parasite transmission levels.

#### **2.5 Complementary Methods in Studying Recrudescence**

##### ***Skin Biopsy in Sentinel Communities***

Some foci have established sentinel villages that have had serial longitudinal evaluations for mf in skin, eyes and nodules during the treatment phase of the elimination program. For this reason, the PCC recommends that these sentinel villages be re-examined by skin snip survey (using microscopy) in the last year of the PTS period, if results of the PCR at that time are  $\geq 1/2000$  and the ATP calculation is  $>20$ . Ophthalmological evaluations are not deemed necessary by the PCC.

### **Nodule Surveillance**

Due to the nonspecificity of subcutaneous nodules in areas where onchocerciasis prevalence is low, the PCC does not recommend nodule surveillance during PTS. If countries elect to implement nodule surveillance, the contents of suspicious masses should be determined by histology (e.g., by resection of the mass, sectioning and staining) and initial finding of *O. volvulus* worms on microscopic examination confirmed by a recognized expert, in consultation with OEPA. (see Annex 4) Alternatively, a more rapid and equally specific methodology could be needle aspiration of a suspected nodule to obtain fluid for PCR testing in a suitable laboratory, again in consultation with OEPA

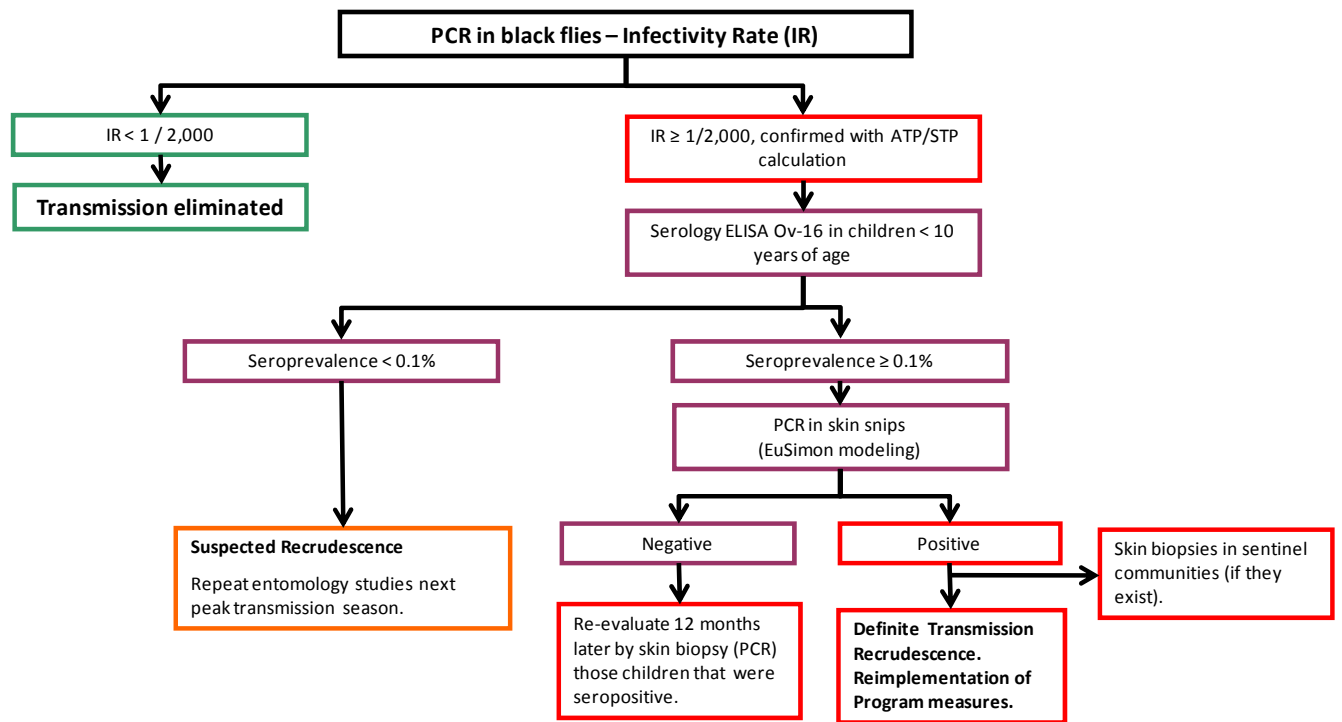
### **2.6 Decision Tree Illustrating PTS Process**

- If in the PCR entomological evaluation there are no positive head pools or the infectivity rate is  $< 1/2000$  and the ATP/STP is  $< 20$ , then it is confirmed that transmission has been eliminated. Nevertheless, the PCC/OEPA could recommend extending PTS in some areas in relation to particular epidemiological and vector considerations.
- Where there are cases of PCR head pools found to be positive where the infectivity rate is  $\geq 1/2000$  (95% C.I.) and the ATP/STP is  $> 20$ , then a serological survey should be undertaken using a sample of 3000 resident children  $< 10$  years of age (if that number of children with whose characteristics were available) with the goal of determining their seropositivity (i.e., the presence of IgG4 specific antibodies against the recombinant antigen Ov-16, in capillary blood drawn from the children).
- If the serological survey yields an antibody prevalence of  $< 1/1000$  or  $< 0.1\%$  (95% C.I.), the results indicate a suspected recrudescence and it is recommended that entomological evaluations be repeated during the next period of peak transmission.
- If the serological survey yields an antibody prevalence of  $\geq 1/1000$  or  $\geq 0.1\%$  (95% C.I.), then there is the possibility of a recrudescence event. To confirm it, skin biopsies should be obtained from seropositive children and the biopsies processed via PCR.
- If PCR results confirm the positive serological results then it is considered that the children are infected and that there is a definite recrudescence in transmission. The families of the children found to be positive should be interviewed to exclude the possibility of travel to other endemic areas or exposure to the vectors. This type of situation will need to be managed on a case by case basis in consultation with OEPA.
- The PCC additionally recommends a re-evaluation via skin biopsy of the sentinel villages, if they were to exist. (see Annex 5)

- If the seropositive children have negative PCR analyzed skin biopsies, they should be considered negative for *O. volvulus* infection. In this situation, recrudescence of transmission is not confirmed but could be occurring. Consequently, these children should be re-examined in 12 months time via skin biopsy, again processed by PCR, so as to confirm their status. This second result should be considered definitive.

Immediately below is the decision tree / flow chart that illustrates the decisions described (see Figure 2):

**Figure 2. PTS Decision Tree for the Detection, Confirmation, and Response to a PRE**



### 2.7 What to Do in Case of Detecting a Potential Recrudescence?

Should entomological and serological evidence indicate that recrudescence has occurred in a particular focus (see Figure 2), then appropriate measures should be initiated to prevent further infections and, where possible, to eliminate the parasite or reduce transmission levels below the maintenance threshold. The PCC requests that countries contact it immediately so that a flexible solution can be determined using state-of-the-art methods specific to the affected focus. This may include vector control, increased treatments with ivermectin, nodulectomy, and use of doxycycline.

### **Component 3: Preparation of a country report in support of the request for PAHO/WHO certification of elimination**

During the PTS period, the national programs will not only have to endeavor in the two components already mentioned (Education and Evaluations) but will also need to dedicate efforts in the organization of data and the preparation of the report that will accompany the request for certification. This report should meet the requisites established by PAHO/WHO in Appendices II, 'Directives for the Preparation of a Country Report', of the guide to criteria for certification (WHO, 2001). WHO, and well as OEPA, are ready to assist in the elaboration of this report.

#### **Certification of elimination request to PAHO/WHO, by country**

Requests to WHO for certification of elimination are not made by focus, but by country. The PCC and OEPA will assist country programs in the process leading up to a request for certification. When the final focus in a given country enters its PTS period, then by definition (in the terminology of WHO certification guidelines), the country has entered the "precertification period" after which a national request for formal certification procedures can be made to PAHO/WHO.

## Annexes

### Annex 1: Schedule of Activities towards the Elimination of Onchocerciasis

The entire approach to certification of onchocerciasis elimination in the Americas is outlined in the following table, with the second major box related to the PTS period.

|                                     |  |
|-------------------------------------|--|
| <b>Treatment phase</b>              | <b>Treatments started</b>  |
|                                     | <ul style="list-style-type: none"> <li>– Baseline and programmatic evaluations (every four years) showing positivity of major indicators (clinical [nodules] parasitological [mf], ophthalmological [MfAC, MfC], and entomological [L3]).</li> </ul>   |
|                                     | <b>Transmission ongoing</b>  |
|                                     | <ul style="list-style-type: none"> <li>– Infective stage <i>O. volvulus</i> larvae found in vectors (heads) and children &lt; 5 years old with Mf in skin, nodules, and serologically positive.</li> </ul>   |
|                                     | <b>Transmission suppressed</b>   |
|                                     | <ul style="list-style-type: none"> <li>– Evaluations begin to show negative results.</li> <li>– Evaluations are conducted oriented to demonstrate that transmission has been interrupted in the focus.</li> </ul>  |
|                                     | <b>Transmission interrupted</b>  |
|                                     | <ul style="list-style-type: none"> <li>– Transmission considered interrupted following negative entomological results.</li> <li>– Complete documentation to corroborate negative results.</li> <li>– PCC's review with government representatives available entomological data.</li> <li>– PCC issues written recommendation to government to stop treatment.</li> <li>– Government accepts recommendations.</li> </ul>  |
| <b>Post Treatment Surveillance</b>  | <b>Transmission interrupted</b>  |
|                                     | <ul style="list-style-type: none"> <li>– Program conducts health education about the reason for halting treatments within the respective focus area</li> <li>– Post treatment surveillance (PTS) implemented for a three year period and evaluations conducted.</li> <li>– PCC reviews results from evaluations corresponding to the PTS period.</li> <li>– New confirmation (recommendation) that elimination has occurred if data support this recommendation.</li> <li>– When elimination is confirmed in the last focus in the country, PCC recommends that the government request initiation of the PAHO/WHO certification process.</li> <li>– OEPA assists country in developing dossier to submit to PAHO/WHO for the certification process.</li> <li>– Results leading to 'stop treatment' decision are published in a peer reviewed journal.</li> </ul> |
| <b>Certification of Elimination</b> | <b>Transmission eliminated</b>   |
|                                     | <ul style="list-style-type: none"> <li>– Country to formally write PAHO/WHO and request certification.</li> <li>– PAHO/WHO forms an independent international team to review the data in the field.</li> <li>– PAHO/WHO grants final certification (whole country only).</li> </ul>  |
|                                     | <b>Final certification of Elimination of Transmission by WHO</b>   |

## **Annex 2: Entomologic Evaluation**

There are diverse considerations in preparing for the entomological evaluation including the composition of the capture team (a collector and an attractant), the elaboration of a calendar in accordance to the period of peak transmission, and the acquisition of materials necessary for the packaging and conservation of the collected flies.

### *Selection of Communities for Evaluation*

The communities to be evaluated are those that have served as sentinel and extra-sentinel communities which is where In-Depth Epidemiological Evaluations (EEP) have taken place. The EEPs in these communities have permitted the evaluation of the impact of mass treatment with ivermectin on transmission and will, in the end, demonstrate that transmission has been interrupted.

### *Selection of Capture Sites within the Community*

In each of the communities to be evaluated, the capture sites should be identified and should be the same sites used during the EEPs.

### *Selection of Season and Hours of Collection*

Transmission seasons can vary by focus. For example, transmission of *O. volvulus* in Guatemala occurs annually between November and April, thus making these the best months for captures. In addition, the greatest numbers of flies are found between 12 noon and 5 pm, so that daily captures should be made during these hours.

The number of collection days depends on the known biting density for the community. If biting rates are low then it would be necessary to collect flies over a greater number of days to assure a sufficient total number of flies collected (about 10,000 flies), and in that way making for a more precise ATP.

### *Collection Procedures*

- Standard methods to assess the biting rate and collect vector specimens should be followed. Each team consists of a collector and an attractant.
- Each national program has standardized their hours for fly collecting in relation to the highest number of parous flies (e.g., from 8 am to 5 pm, from 11 am to 5 pm, etc.).
- Flies are collected for 50 minutes each hour, by aspiration, and before they have a chance to take blood.
- Collected flies are stored in absolute ethanol in tubes labeled with the hour, date, site, and community.
- At the end of each day, flies are separated according to species using a stereoscope.
- The numbers of the vector in the area are recorded for each hour.

### *Laboratory Analysis*

For the PCR analysis of the collected flies, they should be placed in a tube or a jar with no more than 50 flies each.

The heads and bodies of the flies are separated using standard procedures. A representative sample is tested by polymerase chain reaction (PCR) to detect *O. volvulus* DNA. Body pools are analyzed first; if any of the body pools are positive, testing of putative positives is repeated. If the positive body pool is confirmed then body pool testing is suspended and all of the head pools are then analyzed. As part of a process to standardize this procedure, positive controls obtained from the University of South Florida are used. Positives are confirmed by a second PCR.

#### *Data Analysis*

The geometric mean number of vectors caught per hour is calculated as  $\left[\exp\left(\frac{\sum \log(x+1)}{n}\right) - 1\right] / 0.833$ , where  $x+1$  is the number of flies caught in a 50 minute collection period plus 1 (to avoid  $\log[0]$ ),  $n$  is the number of collection periods and 0.833 is the conversion factor to convert a 50 minute collection period into 1 hour. This geometric mean hourly landing rate (which approximates the biting rate, as it will be called hereafter) is calculated for the vector over the capture period. The total biting density for this period (called the seasonal biting density [SBD]) is calculated as the geometric mean hourly biting rate multiplied by 10 potential hours of biting per day and the number of days in the season.

The Poolscreen® program version 2.0 employs a statistical model to calculate the probability of infection of an individual black fly from the number of positive pools and the size of the pools, and will be used to calculate the proportion of infective flies with 95% confidence intervals (CI) computed using the Bayesian method.

The seasonal transmission potential (STP) is calculated as the product of the SBD, the proportion of flies with infective-stage *O. volvulus* larvae and the mean number of infective larvae per infective fly (assumed to be 1 in an area of low transmission). The STP may be equal to, or slightly less than, the annual transmission potential (ATP).

The criteria used by the former Onchocerciasis Control Program of West Africa (OCP) (Cupp 2010) and in recent APOC/Gates-supported evaluations of transmission interruption in West Africa (Mali and Senegal) (Diawara 2009) are a prevalence of infective flies below 0.1% in parous flies, or a prevalence less than 0.05% in all flies (assuming a parity rate of 50%). The sample size required to exclude a prevalence of infective flies of 0.05% in all flies at a 95% confidence level, given that no infective fly is found, is roughly 6,000. This metric differs from the original WHO criterion that called for sampling 10,000 flies. In cases where collections are unable to reach minimum sample size despite collections over the entire transmission season, the ATP or STP is critical to assessing the status of onchocerciasis transmission.

An OEPA-convened meeting of entomologists in September 2006 recommended the use of the ATP or STP although there was controversy surrounding the levels at which transmission breakpoints occur. All entomologists at that meeting agreed that an ATP >20 represented ongoing transmission, and under 5 represented interrupted transmission. However, the ATP or STP below which the reproduction ratio ( $R_0$ ) of the parasite is <1, *i.e.* the threshold transmission potential that indicates the parasite population is moving towards eradication, has yet to be

identified and is likely to vary according to characteristics of the vector species. In actuality, estimates of this threshold transmission potential have ranged from 5 to 54  $L_3$ s/person/year using mathematical models, from 7.6 to 18 using field observations and in general a range of 5 to 20 is considered acceptable by most entomologists.

### **Annex 3: Serologic Evaluation**

The objective is to measure the prevalence of IgG4 antibodies to Ov-16, a recombinant pre-patent antigen of *O. volvulus*, in children under ten years of age.

To determine the population to be included in the serological evaluations, two methods have been employed in the region, each drawing their sample from a differing frame of reference:

1. Includes only endemic communities that had received treatment, and
2. Includes communities that are potentially endemic (that had or had not received treatment).

#### *1. Method that includes only endemic communities under ivermectin treatment*

- A census is made of all children < 10 years of age in each community.
- A list of these communities is made with the number of children < 10 years of age for each one.
- If the total number of children in the focus communities is more than 3,000, then a sample is taken.
- For this, all communities in the focus are considered and a random sample of these communities made for inclusion in the evaluation.

#### *2. Method that includes Potentially Endemic Communities*

This method of sampling in schools was used by Lindblade (2007) in Guatemala in relation to foci with a large number of communities initially identified as potentially endemic but which eventually were excluded from treatment. This fact made it necessary to include these communities in the evaluations to determine an interruption of transmission

#### *Identification of Potentially Endemic Communities (PEC)*

Using historical onchocerciasis transmission maps, a list of communities having at least one of the following characteristics should be compiled:

- a) Past evidence of onchocerciasis transmission (defined as the documented presence of mf in skin or eye, or confirmed onchocercomas in at least one community resident);
- b) Suspicion of past transmission (a survey having taken place, but no positive residents were found); or
- c) History of having been under mass drug administration (MDA) with ivermectin.

The communities that satisfy these criteria are termed “potentially endemic communities” (PECs) and form the basis for sample selection for serological assessments.

If the estimated number of participants was below the targeted sample size of 3,000, then all the children of eligible age in the area should be included.

Efforts should be made to find absent children to ensure maximum participation in the evaluation.

### *Procedures*

Sterile procedures are used to prick the fingers of all participants and 4-6 drops of blood (80-120  $\mu$ L) are absorbed onto Whatman No. 2 filter paper.

The filter paper blood samples are dried, separated by sheets of paper, and then bundled and stored in sealed plastic bags in a cooler until they are returned to the laboratory where they are stored at 4°C, if immediately processed. If processing is not to take place within a short period of time, then the samples should be stored at -20°C.

### *Laboratory Analysis*

Two 6 mm punches of blood-saturated filter paper are placed in a PBS-Tween 0.05% and BSA 5% buffer and eluted overnight at 4°C. The elution is then run in duplicate in a standard enzyme-linked immunosorbent assay (ELISA) to detect IgG4 antibodies against the Ov-16 recombinant antigen. A standard curve is used on each plate to identify positive samples and permit comparisons between plates and over days. Any positive results are repeated before being reported as positive.

#### **Annex 4: Nodule surveillance**

Some programs have elected to undertake nodule assessments during PTS. After a series of discussions about the merit of such surveillance, PCC decided not to endorse nodule surveillance activities, since a number of conditions could give rise to subcutaneous masses that are clinically suspicious for onchocercomas. Thus, false positives can easily result, and care must be given to distinguish between the following categories:

1. "Masses": as reported by untrained personnel or by patients themselves, that often are not clinically suspicious for onchocercomas;
2. "Suspicious masses": clinically resembling onchocercomas; and
3. "Onchocercomas": confirmed histologically or by PCR using fluid drawn from the nodule.

The process that has generally been followed includes:

- Educational activities in coordination with health teams and communities to allow them to report the appearance of any suspicious subcutaneous mass that could be an onchocercal nodule.
- Each 'subcutaneous mass' should be documented as to its clinical details (size, pain, consistency, anatomical location, mobility) and if the mass appeared after treatment was suspended.
- Such masses must be identified first by trained staff from the program to distinguish them from "suspicious masses".
- Likely onchocercomas are then surgically removed and submitted for proper histopathologic testing, in consultation with OEPA, for confirmation.

Active nodule surveillance has been a routine activity in some programs (especially Mexico) and so this mode of field work will undoubtedly continue during the PTS period. However, outside of Mexico, it is assumed that the search of nodules will be mostly passive, with self reporting of masses, or examination by inexperienced health personnel.

## **Annex 5: Sentinel villages**

In its initial phase, OEPA established a methodology to determine the impact of mass treatment with ivermectin in endemic communities, which included the selection of a group of *sentinel* villages or communities that have been subjected to special follow-up activities and to the periodical holding of In-Depth Epidemiological Evaluations (EEP). It is important to point out that not all 13 foci in the region established sentinel communities given the fact that not all foci contained hyper endemic communities. However, as OEPA advanced in the process of elimination, additional communities were selected in areas previously un-evaluated. These communities are known as extra-sentinel communities.

- Sentinel communities were chosen by each national program at the beginning of their operations and the majority of these were hyper endemic (with a baseline prevalence of > 60%). The extra-sentinel communities were chosen using the same criteria. In-depth epidemiological evaluations took place at regular intervals (programmatic impact evaluations); first before treatment started, then after two years and finally at 4-year intervals thereafter. In the extra-sentinel communities, EEPs have also been carried out but not at previously established intervals.
- The evaluations include parasitological (mf and nodules), ophthalmological, serological and entomological indicators.
- Entomological evaluation will be carried out in sentinel and extra-sentinel communities during the PTS period to determine the interruption of transmission.
- The PCC recommends that only skin snip surveys be done in sentinel communities under the following circumstances:
  - The entomological evaluations conducted during the PTS period showed positive results (infectivity rate of  $\geq 1/2000$  and ATP > 20)
  - The serological evaluations demonstrate an IgG4 antibody prevalence against *O. volvulus* of > 0.1%
  - PCR results from biopsies taken from serologically positive children are positive.

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